

TITLE: Standard operating procedure (SOP) for the start-up, maintenance, acquisition, and shutdown of the MACSQuant™ Tyto cell sorter.

EFFECTIVE DATE: 21st February, 2026

ORIGINATED BY: Bethany Carter **(Signature)** **(Date)**

REVIEWED BY: Jodi Kroeger **(Signature)** **(Date)**

I. PURPOSE

This SOP provides standardized instructions for startup, quality control (QC), workspace creation, sorting, data export, and shutdown of the MACSQuant™ Tyto Cell Sorter (Miltenyi Biotec). This procedure applies to all users operating the MQ Tyto cell sorter.

II. REFERENCES

1. Miltenyi Biotec. (2024). *MACSQuant® Tyto® Instrument short instructions: Photomultiplier tube (PMT) calibration* (140-007-180-001.01). Miltenyi Biotec B.V. & Co. KG.
2. Miltenyi Biotec. (2023). *MACSQuant® Tyto® Cell Sorter short instructions: Setting up a sort workspace* (140-006-417.03). Miltenyi Biotec B.V. & Co. KG.
3. Miltenyi Biotec. (2023). *MACSQuant® Tyto® Cell Sorter short instructions: Cartridge priming and sample loading* (140-006-547.01). Miltenyi Biotec B.V. & Co. KG.

III. MATERIALS AND REAGENTS

3.1 Instruments & Hardware

- MACSQuant Tyto (130-103-931)
- MACSQuant Tyto Priming Fixture (130-119-832)

3.2 Buffers & Reagents

- MACSQuant Tyto Running Buffer (130-107-206)
- MACS GMP PBS/MgCl₂ Buffer (170-076-155)
- MACS GMP Tytonase 5 mL (170-076-210)
- Optional: Comparable custom user-prepared buffers and supplements.

3.3 Consumables

- Pre-Separation Filters (20 µm) (130-101-812)
- Standard/ High Speed Cartridges (130-104-791, 130-121-549)
- MACSQuant Tyto Calibration Beads (130-122-730)
- 10 mL syringe with Luer-Lock tip
- Thin-stem transfer pipets, sterile.

Reagent and instrument requirements

Component	Order no.
MACSQuant Tyto	130-103-931
MACSQuant Tyto Priming Fixture	130-119-832
MACSQuant Tyto Running Buffer	
Research grade	
MACSQuant Tyto Running Buffer 1×100 mL	130-107-206
MACSQuant Tyto Running Buffer 6×100 mL	130-107-207
GMP-compliant	
MACS GMP Tyto Running Buffer consisting of	
MACS GMP PBS/MgCl ₂ Buffer 3×1 L	170-076-155
MACS GMP Tytonase 5 mL	170-076-210
Pre-Separation Filters (20 µm)	130-101-812
10 mL syringe with Luer-Lock tip	
Capillary pipet tips, sterile	

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IV. PROCEDURE

4.1. Startup & Quality Control (QC)

1. Tap the lower touch-screen to power on the instrument.
2. Launch the **Tyto** software.
3. Log in using posted username and password.
4. Allow the instrument to warm up for **30 minutes**.
5. Retrieve the pre-loaded QC cartridge and calibration beads from the FCC refrigerator.
6. If necessary, remake the beads according to the following manufacturer protocol:
 - 3 drops MACSQuant Tyto Calibration Beads (130-122-730)
 - 1.5 mL MACSQuant Tyto Running Buffer (130-107-206) or filtered 1x PBS
7. Scan and load the QC cartridge into the instrument.
8. Click the **QC** icon in the upper right corner of the software.
9. When prompted, scan the bead vial.
10. Select '**Start**' to begin QC.
11. When QC completes, the QC report will automatically export to a **Run** report.
12. Confirm that CVs, MFIs, and channel gain approximate expected values.

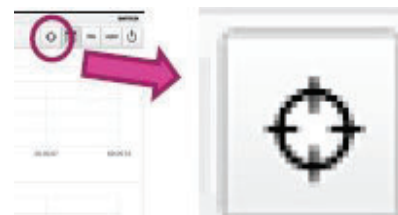


Image courtesy of Miltenyi Biotec

4.2. Cartridge Preparation

Note: It is recommended to utilize the **Automatic Priming** feature on the instrument to avoid damage to the cartridge microchip. Instructions for manual priming are included in this section.

Sample Loading Sample Requirements:

- Volume: 100 μ L – 10 mL
- Maximum cell density: 5×10^7 cells/mL
- Buffer: MACSQuant® Tyto® Running Buffer

Manual Cartridge Priming

Note: If using Automatic Priming, proceed to Sample Loading section.

1. Place the cartridge into the MACSQuant® Tyto® Priming Fixture by sliding the cartridge feet into the slots. Ensure the cartridge is level.
2. Remove the cap from the input chamber.
3. Using a pipette, add MACSQuant® Tyto® Running Buffer to the input chamber until the buffer reaches the propeller (~500 μ L).

4. Priming the positive chamber.
 - Pull the plunger of a 10 mL Luer-Lock syringe to the stop, then attach it to the input chamber.
 - Press the plunger while gently pressing the cartridge. Continue until buffer becomes visible in the **positive collection chamber**.
5. Priming the negative chamber
 - Remove the cartridge from the priming fixture.
 - Block the **pressure inlet with O-ring** using your finger.
 - Push the plunger completely to move buffer into the **negative collection chamber** until the buffer level is just above the 3D filter.

Sample Loading

Note: Remove cartridge from priming fixture during sample loading.

1. Remove the plunger from a 10 mL Luer-Lock syringe; keep the plunger upright.
2. Remove the input chamber cap and attach the syringe body.
3. Place a 20 µm pre-separation filter (130-101-812) on the syringe.
4. Filter sample to remove clumps. Be careful not to over fill the syringe.
5. Discard the filter and save an aliquot as the "original fraction" for analysis.
6. Place the plunger gently onto the syringe.
7. Gently push sample into the cartridge.

Note: Too much pressure may force cells into the negative chamber or rupture the microfluidics tract.

8. Remove the syringe and cap the input chamber.

4.3. Workspace Creation & Sorting

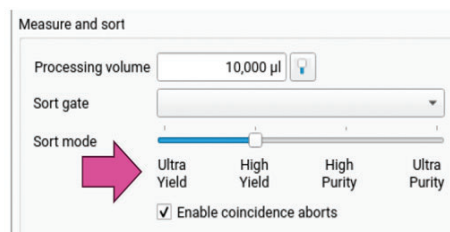
1. To open an existing workspace:
File → Open → Select workspace → Open
2. To create a new workspace, select New Workspace.
 - Name detectors according to markers and fluorophores.
 - Select the scatter plot icon and choose the number of plots needed.
 - Create a morphology gate using the polygon gating tool.
 - Double-click to complete the gate.
 - Right-click the gate to name it.
3. To create a gating hierarchy:
 - Select the parent gate from the dropdown on a blank plot.
 - Adjust axes and draw the new gate.
 - Repeat until the gating strategy is complete.

Flow Cytometry Core, MRC 1295
Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33612

Standard Operating Procedure

4. Select the desired gate from the left panel or right-click and choose **Make Sort Gate**.
5. Choose the desired purity mode.

Note: For large cell cartridges, use **High Yield mode** with **coincidence aborts disabled**.



6. Scan prepared cartridge and load it into the instrument.

Image courtesy of Miltenyi Biotec

7. If the cartridge has not been manually primed, ensure **Auto Prime** is checked.
8. Adjust voltages and backscatter as needed.
9. Save all changes to the template.
10. If desired, transfer volume from the negative chamber back to the input chamber.
11. Reload the cartridge and select **Sort**.

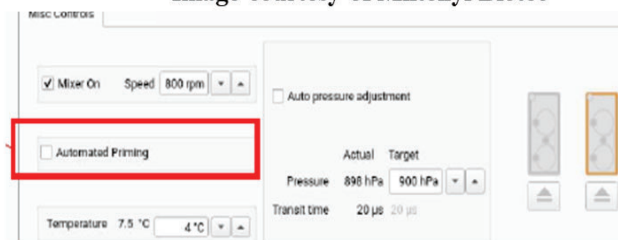


Image courtesy of Miltenyi Biotec

4.4. Exporting Data Files

1. On the bridge computer (mounted next to the Tyto), log in using:
 - Username: flowlab
 - Password: flowlab
2. Locate the **Flowlab** directory and the **Tyto Files** folder.
3. On the Tyto computer:
File → Copy → Data Files → Public
4. From the dropdown, select **Network Share**.
5. Enter the domain password: flowlab
6. Export .mqd files:
 - Select Data Files.
 - Navigate to your experiment.
 - Select 2–3 files per sample.
 - Click Copy.
7. Export Run Report PDFs
 - Select Report Files.
 - Select all run report PDFs for your experiment.
 - Export using the same steps as above.
8. On the bridge computer, open the Tyto Files folder.
9. Open the flowlab directory in a separate window.

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10. If prompted, log in with your Moffitt ID and password.
11. Navigate to your user folder.
12. Drag and drop files from Tyto Files into your user directory.

4.5 Shutdown Procedure

1. Remove the cartridge from the instrument.
2. Save all workspace changes.
3. Select the power button in the upper right corner of the screen. This shuts down both the software and the instrument.

4.6 Troubleshooting

Potential Issues		
Issue	Possible Cause	Corrective Action
<u>Buffer does not appear in positive chamber during manual priming.</u>	<u>Incomplete seal or insufficient pressure</u>	<u>Re-seat syringe; gently press cartridge and retry</u>
<u>Cells appear in negative chamber during sample loading.</u>	<u>Loading rate too high</u>	<u>Reduce to ≤ 0.5 mL/s</u>
<u>Air bubbles in chambers</u>	<u>Incomplete priming</u>	<u>Repeat priming steps</u>
<u>Instrument stops sorting prior to completion</u>	<u>Build up of back pressure</u>	<u>Remove some of the volume in the negative chamber</u>
<u>Cartridge fails to align</u>	<u>Cartridge was loaded incorrectly</u>	<u>Remove the cartridge and reinsert it</u>
	<u>Microchip is damaged</u>	<u>Reload cells into a new cartridge.</u>
<u>Event rate drops to zero</u>	<u>3D filter is clogged</u>	<u>Reload sample into a new cartridge. Adjust sample concentration if necessary</u>

V. REVISION HISTORY

21st Feb, 2026
5th Mar, 2026